Reference: *Biol Bull* 189: 106–112. (October/November, 1995)

Coexistence and Possible Parasitism of Somatic and Germ Cell Lines in Chimeras of the Colonial Urochordate *Botryllus schlosseri*

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Abstract. Fusion between conspecifics (chimerism) is a well-documented phenomenon in a variety of taxa. Chimerism and the subsequent mixing of genetically different stem cell lines may lead to competition between cell lineages for positions in the germ line and to somatic and germ cell parasitism. It is suggested that somatic compatibility systems evolved to alleviate the costs and the threat of such cell lineage competition. Allogeneic colonies of the ascidian *Botryllus schlosseri* form vascular chimeras based on matching in one or both alleles on one highly polymorphic fusibility haplotype. Thereafter, one of the partners is completely or partially resorbed. Here we used a polymorphic molecular marker (PCR typing at a microsatellite locus) to follow somatic and gametic consequences of chimera formation. Twenty-two chimeras and subclone samples were established from 12 different genotype combinations, in which blood cells, zooids, and gonads were typed 45–130 days thereafter. Somatic coexistence of both partners was recorded in 73% of the subcloned chimeras (83% of chimeric entities) up to 100 days after disconnection between genotypes and in all chimeras where colony-resorption was completed. Both genotypes were present in 23% of the sampled gonads (in 33% of the chimeras), and in 22% of the cases, germ cells of the second partner only were detected. Injection of allogeneic but compatible blood cells into three recipient colonies revealed proliferation of the donor cells in one case, 100 days after injection. To further evaluate somatic and germ cell parasitism in chimeric organisms, we propose four key features that characterize cell lineage competition processes. These include the somatic embryogenesis mode of development, the capability for independent existence of stem cells, the disproportionate share of gametic output within chimeras, and the existence of hierarchial responses.

Received 16 March 1995; accepted 27 July 1995.

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Introduction

Genetically non-homogeneous organisms may be established through somatic mutations or via chimerism, the fusion between genetically distinct conspecifics. While most somatic mutation variants are evolutionarily irrelevant (Van Valen, 1988), chimeric entities have been documented in nature from a variety of protists, plants, and animals, belonging to at least nine phyla (Buss, 1982). Several studies (Buss, 1982; Grosberg and Quinn, 1986; Rinkevich and Weissman, 1987a, 1992a) have discussed the evolutionary significance of these natural chimeras by evaluating the fitness costs and benefits of chimerism as compared to the state of genetically homogeneous entities. While several sets of benefits were attributed to natural chimerism (Buss, 1982; Grosberg and Quinn, 1986), particular attention has been paid to the potential costs resulting from the mixing of genetically distinct cell lines within chimeras. This is relevant especially in those cases where germ line sequestration remains undetermined until late in ontogeny, or is never accomplished during the lifespan of an organism (Buss, 1982, 1983; Grosberg and Quinn, 1986; Rinkevich and Weissman, 1987a, 1992a). Within the shared morphological and physiological environments of chimeric entities, one genotype could gain a disproportionate share of germ cells at the expense of the other partner (germ cell parasitism), or one genotype may use the tissues and energy reservoirs of the other

member in the chimera for general maintenance (somatic cell parasitism). Examples on the morphological, cellular, and biochemical levels of the displacement of one cell lineage in the chimera at the expense of another were documented in several groups of organisms (reviewed in Rinkevich and Weissman, 1987a; Grosberg, 1988). However, there is still no direct documentation for such a process in which molecular markers were used.

A typical colony of the urochordate *Botryllus schlosseri*, a subtidal species that can be found worldwide, is composed of a few to several hundreds of modular units called zooids, which are arranged in star-shaped structures (systems) and are connected to each other via a ramified blood system. In this group of organisms, a line of stem cells retains the ability to differentiate into either germ cells or somatic tissue throughout the lifespan of the colony (Berrill and Liu, 1948), continuously replacing aging differentiated cells. Consequently, all zooids within a single colony are genetically identical, having been derived by blastogenesis from a single founder zooid, by a complex but highly synchronized weekly developmental cycle. Zooids and blood vessels are embedded within the tunic, a translucent organic matrix, which bears sausage-like termini of blood vessels, called ampullae, in the colony's periphery. When a colony is split in nature or experimentally into two or more fragments, each subclone usually continues to grow independently to form a larger colony.

Recent interest in botryllid ascidians has centered on allogeneic recognition and its consequences. Pairs of colonies that meet naturally in the wild or are placed in contact under laboratory conditions either fuse their contacting peripheral ampullae to form a vascular parabiont (cytomictical chimera; Rinkevich and Weissman, 1987a), or develop cytotoxic lesions in the contact zone (reviewed in Taneda *et al.*, 1985; Weissman *et al.*, 1990; Rinkevich, 1992). This allorecognition is genetically controlled by a single, highly polymorphic, fusibility/histocompatibility (Fu/HC; Weissman *et al.*, 1990) haplotype with multiple codominantly expressed alleles. Fusion may be established between genotypes which match in one or both Fu/HC alleles. Rejecting colonies share no Fu/HC alleles (Scofield *et al.*, 1982). Controlled laboratory experiments on *Botryllus* chimeras revealed that the zooids from one genotype in each specific chimera are all morphologically eliminated within a few days to several months by massive phagocytosis, leaving the zooids of the other partner intact (Rinkevich and Weissman, 1987a, b, 1992a, b; Weissman *et al.*, 1990; Rinkevich *et al.*, 1993). This phenomenon, called "colony resorption" (Rinkevich and Weissman, 1987b), typically occurs at the end of a blastogenic cycle and appears to be controlled genetically by a multilevel hierarchial organization of histocompatibility alleles (Rinkevich, 1993; Rinkevich *et al.*, 1993). In addition to this resorption phenomenon, a few studies have documented, within chimeras, germ cell transfer and establishment between different partners (Sabbadin and Zaniolo, 1979; Rinkevich and Weissman, 1987a). These preliminary results further indicate that chimerism may present substantial fitness costs over the long term through cell lineage competition, parasitism, or a combination of both processes (Buss, 1982; Grosberg and Quinn, 1986; Rinkevich and Weissman, 1987a, 1992a; Sabbadin and Astorri, 1988).

Materials and Methods

Animals

Botryllus schlosseri colonies were cultured in the laboratory as previously described (Boyd *et al.*, 1986; Rinkevich and Weissman, 1987b, 1992a; Rinkevich *et al.*, 1993). Experimental colonies were isolated from among the progeny of our laboratory stock, which originated about a year earlier from Monterey marina, CA, USA. Newly metamorphosed colonies were raised individually, each attached to glass slides (50 × 75 mm).

Colony allorecognition assays

The technique of colony allorecognition assay (CAA) is the most common assay used for revealing self-nonself discrimination potential in botryllid ascidians (Rinkevich, 1992, 1995; Saito *et al.*, 1994). In this assay, small or large groups of zooids at the growing edges of the colonies are isolated by dissecting them from each colony without injuring their surrounding ampullae. Subclones from two colonies are put on glass slides in pairs and placed so that they contact one another with their extending ampullae. They are usually allowed to fasten themselves to the slides by placing them in a moisture chamber for 30–45 min before transferring to the tanks or to the sea. The CAA was performed on all botryllid ascidians where specificity was analyzed. Observations on the contact sites and colonies were made under the dissecting microscope at least once a week to confirm the location of "each partner" in the chimera in accordance with the developing buds during successive blastogenic cycles. A complete mixture of blood-borne pigment cells was recorded <14 days after fusion (Rinkevich and Weissman, 1987b).

Experimental procedures

Polymorphic molecular markers, such as microsatellite loci, may be most suitable for individual identification of genotypes within *B. schlosseri* chimeras. Microsatellites are tandem repeats (usually >100 bp long) of very short nucleotide motifs (1–6 bp long) that are dispersed abundantly and randomly through eukaryotic genomes. Since each of the microsatellites is flanked within the DNA ma-

terial by unique sequences, they can be amplified *in vitro* using the polymerase chain reaction (PCR; Queller *et al.*, 1983). We used the protocol developed for PCR typing at *B. schlosseri* microsatellite locus 811, which has been found to be highly polymorphic in two *Botryllus* populations (Pancer, 1994; Pancer *et al.*, 1994). Twenty-three laboratory-raised colonies were typed at microsatellite 811 and were assigned to their fusibility status by employing colony allorecognition assays (Rinkevich, 1992, 1995; Saito *et al.*, 1994) on different pair combinations.

Samples for typing were either a small tissue fragment (T); or hemolymph (H); or a single zooid (Z), and the gonad (G), if present in that zooid. Gonads are situated on both sides of the zooids, composed of lobulated testes and ovaries. In this study the sperm and the small oocytes from each gonad were sampled as a whole in the PCR reaction. Whenever the state of the chimeras allowed, simultaneous sampling of several zooids was performed. Tissue samples were transferred into 1.5-ml test tubes, rinsed with 0.5 ml filtered (0.2 μM, Schleichter & Schuell) seawater, and were then boiled for 5 min in 100 μl TE, dispersed by pipetting through an aerosol-free tip (EL-KAY, Labsystems) and centrifuged at 12000 $\times$ *g* for 5 min. The supernatant was discarded, the pellet resuspended in 10–20 μl of PCR-lysis buffer (20 m*M* DTT, 10^{-3}% SDS and 0.5 mg/ml Proteinase K) and incubated for 1 h at 55°C with occasional vortexing. At the end the sample was boiled again for 5 min. Amplifications were performed on a PTC-100 thermal cycler (MJ Research) in 10 μl reaction mixtures containing 1 μl of the sample, 4 pmoles each of the forward and reverse primers, 200 μ*M* of each dNTP, 0.1 μCi [α-^{32}P]dCTP, 5% DMSO, 0.5 U *Taq* DNA polymerase and buffer (Boehringer, Mannheim). The cycling parameters were 3 min at 96°C followed by 30 cycles of 45 s at 95°C, 75 s at 60°C, and 15 s at 74°C. The reactions were stopped by the addition of 5 μl Sequenase stop solution (USB), denatured for 5 min at 95°C, then 2 μl of each sample were electrophoresed on a 6% denaturing polyacrylamide gel. Detection threshold was determined experimentally in cell mixtures of 1000 cells total, containing different proportions of two homozygous colonies. Cells of a genotype mixed 1:10–1:20 with cells of a second genotype could be detected following three exposure days of the film (Pancer, 1994).

For microinjections, a siliconized micropipette mounted on a micromanipulator was inserted into a large blood vessel. Five to seven microliters of hemolymph were drawn. The micropipette's contents were then injected into the recipient colony's blood vessels. Several cell counts revealed approximately 2.5–5 $\times$ 10^4 blood cells in that volume.

Results

We studied 22 whole chimeras and chimeric subclones that were established from 12 different genotype combinations (Table I, Fig. 1a–c). Different parts of the colony's body, including blood cells, individual zooids, pieces of tissue, and gonads were sampled 45–130 days after establishment of the chimeras or 15–100 days after disconnections between the partners in the chimera as a result of subcloning, spontaneous disconnection, or after immunological resorption (Table I).

In four genotype combinations (chimeras A, B, C, G, Table I, Fig. 1a–c), we sampled 2–5 subclones from each original chimera at different times following chimera formation or disconnection (experimentally or spontaneously). In one of these chimeras (G) a hierarchial response was documented. Seventy days following chimera formation, genotype BE was recorded in all DD regions sampled (cases 13, 15, 16), while genotype DD was not detected in the BE regions (cases 12, 14). In pair combination C (cases 8, 9) the two subclones varied, since one contained both genotypes in the zooid soma 55 days following fusion, while the second was not detected in the other partner's soma 70 days after chimera formation. Although subclones of pair combinations A and B were not assigned to their pretyped genotypes, it is clear that six out of the seven subclones possessed soma of both genotypes up to 125 days after chimera formation (Table I, Fig. 1a–c). In six (75%) of the remaining eight chimeras, the other partner's soma was detected, together with the resident partner's soma, up to 60 days after fusion. Most interestingly, in all four chimeras where colony resorption was completed (cases 17, 19–21, Table I, Fig. 1c), the resorbed genotype was clearly detected together with the "winner's" genotype, even 35 days after resorption. In summary, both genotypes were recorded in 16 (73%) of the subclones and 10 (83%) of the chimeras (Table I).

In 13 of the cases (9 chimeras) studied, gonads (excluding mature oocytes) were sampled from the same zooids where the soma was typed. In three cases (33% of chimeras, nos. H, J, L, Table I, Fig. 1c) both genotypes were present simultaneously in the gonads. These chimeras were sampled 20–30 days after a complete immunological resorption of one partner in the chimera was recorded (75% of the studied resorptions). In all the nine cases (five chimeras) where the partners were disconnected experimentally or spontaneously, we did not record any chimeric situation in the gonads up to 100 days after separation. However, in two of these cases (nos. 3, 12; Table I, Fig. 1a,c), the gonads were typed as belonging to the other genotype in the chimera while the corresponding zooid's soma was only of the original genotype.

In an additional set of experiments, blood cells were injected from pretyped Fu/HC-compatible allogeneic donors into the blood system of three different recipient colonies (colonies C1–C3, Fig. 1d). The recipient colonies were sampled 20 days after injection and then 30–80 days thereafter (50–100 days after injection). In one case (C3,

Table I

Typing at locus 811 of soma and gonads within B. schlosseri *chimeras, 45–130 days following fusion*

Case no.	Microsatellite typing of chimera partners I:II	Chimera and subclone code[a]	Typing chimeras, following disconnections or resorptions: Typing times (days): Following chimera formation	Typing chimeras, following disconnections or resorptions: Typing times (days): Following disconnection or resorption[b]	Type of[c]: Soma I:II	Type of[c]: Gonads I:II
1	BE:DD	A1	125	95, E	+++:−++	nd
2		A2	45	15, E	+++:−++	nd*
3		A3	130	100, E	−−−:+++	+++:−−−
4	AA:DD	B1	45	15, E	+++:−++	nd*
5		B2	45	15, E	+++:−−+	nd*
6		B3	125	95, E	+++:+++	+++:−−−
7		B4	125	95, E	+++:−−+	+++:−−−
8	AA:DD	C1 [AA]	55	20, E; 40, S	+++:+++	nd*
9		C2 [AA]	70	35, E; 55, S	+++:−−−	nd*
10	AA:BE	D [AA]	45	30, S	+++:+++	nd*
11	AD:DD	E [DD]	45	35, S	−−−:+++	nd*
12	BE:DD	G1 [BE]	70	20, S	+++:−−−	−−−:+++
13		G1 [DD]	70	20, S	+++:+++	nd
14		G2 [BE]	70	50, E	+++:−−−	+++:−−−
15		G3 [DD]	70	50, E	+++:+++	−−−:+++
16		G4 [DD]	70	50, E	+++:+++	−−−:+++
17	AD:BE	H [BE]	60	30, R	+++:+++	+++:+++
18	BE:DD	I [BE]	65	40, S	+++:−−−	+++:−−−
19	AA:CC	J [CC]	55	25, R	+++:+++	−−+:+++
20	AA:BE	K [BE]	55	35, R	+++:+++	−−−:+++
21	AA:CC	L [AA]	50	20, R	+++:+++	+++:+++
22	CC:DD	N [DD]	40	30, S	+++:+++	−−−:+++
Both genotypes recorded in:		subclones			16 (73%)	3 (23%)
		chimeric entities			10 (83%)	3 (33%)
A single genotype recorded in:		subclones			6 (27%)	10 (77%)
		chimeric entities			2 (17%)	6 (67%)
Only the other partner recorded in:		subclones			0 (0%)	2 (15%)
		chimeric entities			0 (0%)	2 (22%)

[a] Twelve chimeras are designated by capital letters (A–N). When applicable, their corresponding subclones are marked with numbers. In brackets: The original resident's typed alleles at locus 811 (not recorded for chimeras A and B). Chimeras A, B refer to Figure 1a, chimera G to Figure 1b, c and chimeras H, J, K, L to Figure 1c.

[b] Time elapsed since disconnections in the chimeras due to subcloning, an experimental manipulating (E); spontaneous disconnection (Rinkevich and Weissman, 1989) between the partners in the chimera (S), or resorption (R) of one partner (Rinkevich and Weissman, 1987b, 1992a, b; Weissman *et al.*, 1990; Rinkevich *et al.*, 1993).

[c] Soma typing was performed from either a tissue fragment or a single zooid. The gonad was from the same zooid sampled for soma. nd = not determined due to non-reproductive state. *Asterisks designate dying colonies, sampled from the last remains. Autoradiographic patterns were digitized into one of four categories: a strong signal on the film after an overnight exposure (+++) or a weak signal (−++); detection only after three exposure days (−−+) or below the detection threshold (−−−).

Fig. 1d), the hemolymph of the recipient unequivocally showed also the injected partner's genotype, which may indicate a proliferative process for the injected blood cells. However, in the sampled zooid and gonads of this recipient, the injected genotype was below the detection threshold.

Discussion

In five of the nine ovigerous chimeras (56%) we documented situations where vascular fusion between allogenic *B. schlosseri* colonies led to free exchange of stem cells (that eventually become germ cells) across the former boundary between the two genotypes and to incubation of foreign germ lines within the gonads. In two of the cases only the other partner's germ cells were detected. The immunological resorption phenomenon (Rinkevich and Weissman, 1987b, 1992a, b; Weissman *et al.*, 1990; Rinkevich *et al.*, 1993), in which one partner in a *Botryllus* chimera is morphologically eliminated by phagocytosis, does not exclude the possibility that the "resorbed genome" will be reestablished and coexist within the "win-

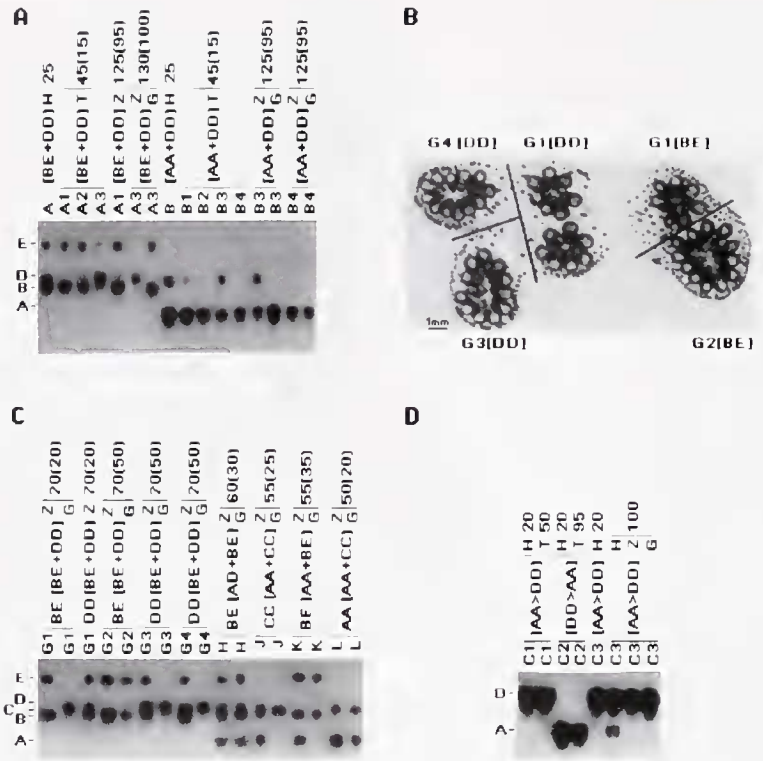

Figure 1. (A) Typing at microsatellite locus 811 of genotype combinations A and B (divided into subclones A1–A3 and B1–B4). Allelic typing (21) of the partners is shown in brackets. Compartments sampled are the hemolymph (H); a tissue fragment (T); a single zooid (Z) and if present, from the gonads (G) of the same zooid. Chimeras were first sampled 25 days after vascular anastomosis, after subcloning (numbers in parentheses denote days after subcloning), and upon sexual maturation. Predetermined alleles in the population (A = 216 bp, B = 232, C = 236, D = 240, E = 268) are depicted at the left side. Horizontal bar-lines group simultaneous sampling of subclones, or samples from several compartments of a single subclone. An overnight exposure of the film is shown. (B) A pictorial outline of genotype combination G depicting the corresponding subclones. The chimera was cut into 4 subclones, 20 days after fusion (marked lines in the figure): G1, the original chimera with three systems; G2, a single system, an outgrowth from genotype BE; G3 and G4, each a single system originating from genotype DD. Thirty days later (50 days after chimera formation) subclone G1 disconnected naturally along the region of fusion, into subclone G1 [BE] and subclone G1 [DD]. The bar line represents 1 mm. (C) Typing at microsatellite locus 811 of genotype combinations G, H, J, K, L. Allelic typing of the partners in each chimera is shown in brackets. Sampling days and compartments as in Figure 1a. Subclone sampling from genotype combination G is as depicted in Figure 1b. Genotype combinations H, J, K, L were sampled after the resorption of one partner in each (numbers are for day after fusion; numbers in parentheses denote days after resorption). An overnight exposure of the film is shown. (D) Typing of three recipient colonies which were microinjected with allogeneic-compatible hemolymph (C1–C3). Only C3 was in good condition, which allowed full screening of: hemolymph (H), a zooid (Z) and the gonad it contained. Sampling of C1 and C2 was from the last remaining tissue fragment (T). Horizontal bars group chronological sampling, or from various sources (C3). A three-day exposure of the film is shown.

ner" partner's body in both the soma (100%) and the gonads (75%). Under these conditions, one genotype in the chimera could parasitize the other (Buss, 1982, 1983; Grosberg and Quinn, 1986; Rinkevich and Weissman, 1987a, 1992a; Grosberg, 1988) by somatic or germ cell parasitism through competitive processes among the genotypes over somatic and gametic positions. The potential for cell lineage competition and parasitism in botryllid ascidians stems from the documentation that these animals maintain self-perpetuating stem cell lineages throughout their lifespan (Berrill and Liu, 1948) as do many other organisms (Buss, 1982, 1983). The displacement of one cell lineage by another in some other groups has been suggested to occur in chimeras of cellular slime molds (Buss, 1982), myxomycetes (Clark and Collins, 1973), fungi (Davis, 1959), sponges (Van de Vyver, 1988; Mukai, 1992), and hydractiniid hydroids (Hauenschild, 1956; Muller, 1964).

The results of the present study do not unequivocally prove somatic and germ cell parasitism. Parasites by def-

inition live at the expense of a host organism (Michalakis *et al.*, 1992). However, parasite/host relationships may reflect a continuum flanked by two extremes. At the one, the parasite may cause the host's death. At the other, the parasite may evolve as "benign." This occurs when parasite and host have a relatively large "shared desiderata list" (Dawkins, 1990). Unfortunately, the literature deals primarily with parasitism by species of distant taxa from the hosts, and there is almost no theoretical treatment to intraspecific parasitism on the cellular level. Therefore, we define germ/somatic cell parasitism in the *Botryllus* chimeric system as follows: 1. Incubation of any number of alien male/female germ cells within gonads. This is especially marked where one genotype's oocytes acquire nutrients from follicle cells (Manni *et al.*, 1994) of another genotype, or compete for space and nutrition (Sabbadin and Zaniolo, 1979) with other developed germ cells. 2. Penetration and establishment of alien somatic cells as an integral part of the resident's soma (zooids), where they can support the development of germ cells. However, in the absence of cell lineage competition, chimeras should show stable sexual expression, with the original germ cells in the appropriate region of the chimera (Sabbadin and Astorri, 1988; Shenk, 1991). When cell lineage competition occurs, four key features may be assigned to characterize the processes involved within chimeric organisms: 1. Cell lineage competition is evolutionarily relevant to taxa developing through the somatic embryogenesis or epigenetic developmental types, where totipotent stem cells are competent to produce germ cells at any point in ontogeny or during most of the lifespan of the organism, respectively (Buss, 1982, 1983; Tuomi and Vuorisalo, 1989). 2. These cell lineages should be capable of increasing in frequency, eventually establishing an independent existence (Buss, 1982). 3. Competing cell lineages in chimeric entities should gain a disproportionate share of gametic output by, for example, restricting the differentiation of the totipotent cells to gametic descendants, while using the somatic constituents of the other partner for maintenance and space provisions for gametes (Buss, 1982, 1983; Buss and Green, 1985; Grosberg, 1988; Sabbadin and Astorri, 1988). 4. Either transitive or nontransitive hierarchial responses for somatic/germ cell parasitism should be established in chimeric combinations from each studied group of compatible genotypes. This also provides further insights into the genetic rules operating for the cell lineage competition processes. Somatic embryogenesis (Berrill and Liu, 1948; Buss, 1982, 1983; Rinkevich and Weissman, 1987a, 1992a; Grosberg, 1988). proliferation of foreign cells in the blood (Fig. 1d; Sabbadin and Zaniolo, 1979), and foreign gametic output (Sabbadin and Zaniolo, 1979; Sabbadin and Astorri, 1988; Fig. 1a–c, Table 1) have already been attributed to *B. schlosseri* chimeras. There is also evidence for a hierarchial relationship for somatic positions in *Botryllus* chimeras (cases 12–16, Table 1, Fig. 1b,c).

In the vast majority of the chimeras (83%) including all chimeras where colony resorption was recorded, and in 73% of all subclones studied, both genotypes were recorded in the soma (Table 1, Fig. 1a–c). One may therefore postulate for the opposite conclusion that these outcomes are characteristic to synergistic relationships between the genotypes within the chimeras in which, for example, each partner in the chimera expresses proportionally its capacities for somatic growth or gametic-product differentiation. In such a case, the chimeric growth/reproductive output should be greater than that of each genotype alone. Previous results, however, showed that neither growth rates nor reproductive activities were improved in *Botryllus* chimeras as compared to single colonies or rejecting partners (Rinkevich and Weissman, 1992a). It should also be taken into consideration that the above results may be the outcome of a chaotic situation within the chimeras in which free circulating germ cell primordia are positioned haphazardly within the developing gonads. This possibility may be critically evaluated by establishing possible hierarchial relationships for gametic positions in *Botryllus* chimeras. The surprising result that even after a complete resorption of one partner in the chimera, the resorbed genotype may continue to thrive, not only in the form of germ cells but also as an integral part of the "winner's" soma, may further suggest that the Fu/HC (Weissman *et al.*, 1990) and the resorption-histocompatibility (Re/HC; Rinkevich, 1993) loci that are very effective in self-nonself discrimination of many already determined cell lineages do not distinguish (or do not activate the effector arm against) the most important group of the stem cells. In such a scenario, the freely circulating stem cells may uninterruptedly differentiate into both gametes and somatic tissues throughout the life span of the original chimera and its corresponding subclones, which are created by splitting the chimera into fragments. Different subclones probably possess unlike proportions of the mixed genotype's stem cells, which may result in the variations observed for the chimeric constituents of both soma and gametes. Therefore, more attention should be paid to possible interactions between different lines of stem cells (such as the displacement of one at the expense of another) rather than to the idea of somatic germ cell parasitism in the context of the evolution of somatic tissue compatibility (Buss, 1982; Buss and Green, 1985; Rinkevich and Weissman, 1987a; Grosberg, 1988).

Within *B. schlosseri* chimeras, transferred cells between genotypes may persist for many blastogeneic generations as circulating elements before maturing as sperm or eggs (Sabbadin and Zaniolo, 1979; Sabbadin and Astorri, 1988). In *B. schlosseri*, therefore, stem cells are probably the level at which natural selection could act, rather than,

as believed, at the colony level (Tuomi and Vuorisalo, 1989). By using molecular markers, such as microsatellites, new discoveries can shed more light on the evolutionary processes that shaped chimerism and the detailed consequences of fusion between compatible allogeneic organisms.

Acknowledgments

We are grateful to R. K. Grosberg and D. Stoner for critically reading the manuscript, to R. Pancer for editing a first draft of the manuscript, and to Elul productions for graphic assistance. This study was supported by a Career Development Award to B.R. from the Israel Cancer Research Foundation—U.S., a grant from the U.S.–Israel Binational Science Foundation, and a grant from S. Price, NAF/IOLR.

Literature Cited

Berrill, N. J., and C. K. Liu. 1948. Germ plasm, Weissmann, and Hydrozoa. *Q. Rev. Biol.* **23:** 124–132.

Boyd, H. C., S. K. Brown, J. A. Harp, and I. L. Weissman. 1986. Growth and sexual maturation of laboratory-cultured Monterey *Botryllus schlosseri*. *Biol. Bull.* **170:** 91–109.

Buss, L. W. 1982. Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Natl. Acad. Sci. USA* **79:** 5337–5341.

Buss, L. W. 1983. Somatic variation and evolution. *Paleobiology* **9:** 12–16.

Buss, L. W., and D. R. Green. 1985. Histoincompatibility in vertebrates: the relict hypothesis. *Dev. Comp. Immunol.* **9:** 191–201.

Clark, J., and O. R. Collins. 1973. Directional cytotoxic reactions between incompatible plasmodia of *Didymium iridis*. *Genetics* **73:** 247–257.

Davis, R. 1959. Asexual selection in *Neurospora crassa*. *Genetics* **44:** 1291–1308.

Dawkins, R. 1990. Parasites, desiderata lists and the paradox of the organism. *Parasitology* **100:** S63–S73.

Grosberg, R. K. 1988. The evolution of allorecognition specificity in clonal invertebrates. *Q. Rev. Biol.* **63:** 377–412.

Grosberg, R. K., and J. F. Quinn. 1986. The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. *Nature* **322:** 456–459.

Hauenschild, C. von. 1956. Uber die Vererbung einer Gewebevertraglichkeits-Eigenschaft bei dem Hydroidpolypen *Hydractinia echinata*. *Z. Naturforsch.* **116:** 132–138.

Manni, L., G. Zaniolo, and P. Burighel. 1994. Ultrastructural study of oogenesis in the compound ascidian *Botryllus schlosseri* (Tunicata). *Acta Zool.* **75:** 101–113.

Michalakis, Y., I. Olivieri, F. Renaud, and M. Raymond. 1992. Pleiotropic action of parasites: How to be good for the host. *TREE* **7:** 59–62.

Mukai, H. 1992. Allogeneic recognition and sex differentiation in chimeras of the freshwater sponge *Ephydatia muelleri*. *J. Exp. Zool.* **237:** 241–255.

Muller, W. 1964. Experimentelle Untersuchungen uber Stockentwicklung, Polypendifferenzierung und Sexualchimaren bei *Hydractinia echinata*. *Wilhelm Roux Archiv* **155:** 181–268.

Pancer, Z. 1994. Somatic and gametic consequences of chimerism in the colonial protochordate *Botryllus schlosseri*. Ph.D. Dissertation, Technion—Israel Inst. Technol.

Pancer, Z., H. Gershon, and B. Rinkevich. 1994. Direct typing of polymorphic microsatellites in the colonial tunicate *Botryllus schlosseri* (Ascidiacea). *Biochem. Biophys. Res. Commun.* **203:** 646–651.

Queller, D. C., J. E. Strassmann, and C. R. Hughes. 1983. Microsatellites and kinship. *TREE* **8:** 285–288.

Rinkevich, B. 1992. Aspects of the incompatibility nature in botryllid ascidians. *Anim. Biol.* **1:** 17–28.

Rinkevich, B. 1993. Immunological resorption in *Botryllus schlosseri* (Tunicata) chimeras is characterized by multilevel hierarchial organization of histocompatibility alleles. A speculative endeavor. *Biol. Bull.* **184:** 342–345.

Rinkevich, B. 1995. Morphologically related allorecognition assays in botryllid ascidians. Pp. 17–21 in *Techniques in Fish Immunology. 4 Immunology and Pathology of Aquatic Invertebrates*, J. S. Stolen, T. C. Fletcher, S. A. Smith, T. J. Zelikoff, S. L. Kaattari, R. S. Anderson, K. Söderhäll, and B. A. Weaks-Perkins, eds. SOS Publications, Fair Haven, NJ.

Rinkevich, B., and I. L. Weissman. 1987a. Chimeras in colonial invertebrates: a synergistic symbiosis or somatic and germ parasitism? *Symbiosis* **4:** 117–134.

Rinkevich, B., and I. L. Weissman. 1987b. A long-term study of fused subclones of a compound ascidian. The resorption phenomenon. *J. Zool. (Lond.)* **213:** 717–733.

Rinkevich, B., and I. L. Weissman. 1989. Variation in the outcomes following chimera formation in the colonial tunicate *Botryllus schlosseri*. *Bull. Mar. Sci.* **45:** 213–227.

Rinkevich, B., and I. L. Weissman. 1992a. Chimeras vs genetically homogeneous individuals: potential fitness costs and benefits. *Oikos* **63:** 119–124.

Rinkevich, B., and I. L. Weissman. 1992b. Allogeneic resorption in colonial proto-chordates—consequences of nonself recognition. *Devel. Comp. Immun.* **16:** 275–286.

Rinkevich, B., Y. Saito, and I. L. Weissman. 1993. A colonial invertebrate species that displays a hierarchy of allorecognition responses. *Biol. Bull.* **184:** 79–86.

Sabbadin, A., and C. Astorri. 1988. Chimeras and histocompatibility in the colonial ascidian *Botryllus schlosseri*. *Dev. Comp. Immunol.* **12:** 737–747.

Sabbadin, A., and G. Zaniolo. 1979. Sexual differentiation and germ cell transfer in the colonial ascidian *Botryllus schlosseri*. *J. Exp. Zool.* **207:** 289–304.

Saito, Y., E. Hirose, and H. Watanabe. 1994. Allorecognition in compound ascidians. *Int. J. Dev. Biol.* **38:** 237–247.

Scofield, V. L., J. M. Schlumpberger, L. A. West, and I. L. Weissman. 1982. Protochordate allorecognition is controlled by an MHC-like gene system. *Nature* **295:** 499–502.

Shenk, M. A. 1991. Allorecognition in the colonial marine hydroid *Hydractinia* (Cnidaria/Hydrozoa). *Am. Zool.* **31:** 549–557.

Taneda, Y., Y. Saito, and H. Watanabe. 1985. Self or nonself discrimination in ascidians. *Zool. Sci.* **2:** 433–442.

Tuomi, J., and T. Vuorisalo. 1989. Hierarchial selection in modular organisms. *TREE* **4:** 209–213.

Van de Vyver, G. 1988. Histocompatibility responses in freshwater sponges: A model for studies of cell-cell interactions in natural populations and experimental systems. Pp. 1–14 in *Invertebrate Historecognition*, R. K. Grosberg, D. Hedgecock, and R. K. Nelson, eds. Plenum, New York.

Van Valen, L. M. 1988. Is somatic selection an evolutionary force? *Evolut. Theory* **8:** 163–167.

Weissman, I. L., Y. Saito, and B. Rinkevich. 1990. Allorecognition histocompatibility in a protochordate species: Is the relationship to MHC semantic or structural? *Immun. Rev.* **113:** 227–241.